

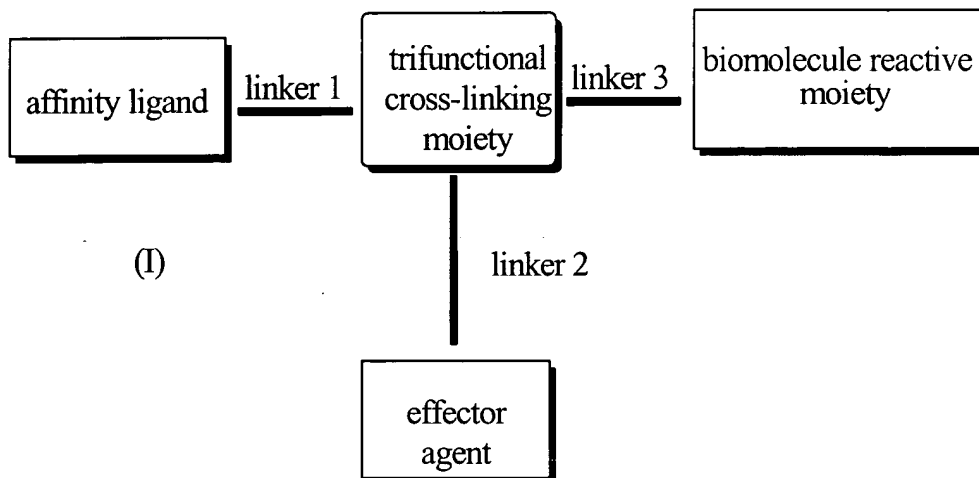
IN THE CLAIMS:

Cancel claims 35, 37, and 39 without prejudice or disclaimer.

Please amend the claims and add new claim 98 as shown below in the LISTING OF CLAIMS.

Claims 1-32 (cancelled)

Claim 33 (Currently Amended): A single molecule reagent for conjugation to a biomolecule, provided with at least three functional parts, and corresponding to the following schematic structure (I) :



the single molecule reagent comprising:

a trifunctional cross-linking moiety, which is optionally tetrafunctional;

an affinity ligand, coupled to the trifunctional cross-linking moiety via a first linker and a biotinamide bond which is has been stabilized to inhibit enzymatic cleavage, by introducing an alpha carboxylate, an aspartic moiety, or an N-methyl group in linker 1, of the affinity ligand such that binding with avidin or streptavidin is not diminished by

sterical hindrance, the affinity ligand comprising biotin or a biotin derivative having essentially the same binding function to avidin or streptavidin as biotin having an affinity to bind specifically to at least one member selected from the group consisting of avidin; a derivation of avidin having essentially the same binding function to the affinity ligand as avidin; streptavidin; and a derivation of streptavidin having essentially the same binding function to the affinity ligand as streptavidin, the chosen member, and said biotin derivative exhibiting an affinity constant of at least ~~10⁶~~ 10⁶ M⁻¹ to avidin or streptavidin toward the affinity ligand;

an effector agent, covalently coupled to the trifunctional cross-linking moiety ~~by a covalent bond~~ either directly or by a second linker, the effector agent having an in vivo, ex vivo or in vitro effect on at least one member selected from the group consisting of a cell, a tissue, and a humoral molecule other than a biomolecule linked to the tri-functional cross-linking moiety, wherein the effector agent is selected from the group consisting of a synthetic or naturally occurring toxin; an enzyme, optionally for converting a pro-drug to an active drug; a hormone; an immunosuppressive agent; an immuno-stimulating agent; a radionuclide binding/bonding moiety to which is optionally bound or chelated a radiosensitizer, an enhancer for an X-ray, MRI or ultrasound technique, or a non-radioactive element which can be converted to a radioactive element by means of external irradiation; a photoactive compound; a compound used in photoimaging; and a compound used in photodynamic therapy; and a biomolecule reactive moiety, coupled to the trifunctional cross-linking moiety, optionally via a third linker, the biomolecule reactive moiety being able to react with a biomolecule to form a covalent bond with the biomolecule.

Claim 34 (Previously Presented): The single molecule reagent according to claim 33, wherein the trifunctional cross-linking moiety comprises a member selected from the

group consisting of triaminobenzene, tricarboxybenzene, dicarboxyaniline and diaminobenzoic acid.

Claim 35 (Canceled)

Claim 36 (Previously Presented): The single molecule reagent according to claim 33, wherein the affinity ligand comprises a biotin derivative selected from the group consisting of norbiotin, homobiotin, oxybiotin, iminobiotin, desthiobiotin, diaminobiotin, biotin sulfoxide, and biotin sulfone.

Claim 37 (Canceled)

Claim 38 (Currently Amended): The single molecule reagent according to claim ~~37~~ 33, wherein the biotin derivative is selected from the group consisting of norbiotin and homobiotin.

Claim 39 (Canceled)

Claim 40 (Currently Amended): The single molecule reagent according to claim ~~35~~ 33, wherein the first linker comprises at least one member selected from the group consisting of compounds containing hydrogen-bonding atoms, and compounds containing ionizable groups, to thereby increase the water solubility of the biotin moiety.

Claim 41 (Previously Presented): The single molecule reagent according to claim 40, wherein the compound containing hydrogenbonding atoms comprises a member selected from the group consisting of ethers and thioethers.

Claim 42 (Withdrawn): The single molecule reagent according to claim 40, wherein the ionizable groups are selected from the group consisting of carboxylates, sulfonates, and ammonium groups.

Claim 43 (Withdrawn): The single molecule reagent according to claim ~~35~~ 33, wherein the first linker comprises an alpha carboxylate, or an N-methyl group, to thereby stabilize a biotinamide bond against enzymatic cleavage by biotinidase.

Claim 44 (Withdrawn): The single molecule reagent according to claim 33, wherein the effector agent comprises an aminocarboxy derivative or a cyclic amine.

Claim 45 (Withdrawn): The single molecule reagent according to claim 44, wherein the aminocarboxy derivative is selected from the group consisting of an EDTA derivative and a DTPA derivatives.

Claim 46 (Withdrawn): The single molecule reagent according to claim 44, wherein the aminocarboxy derivative is selected from the group consisting of Me-DTPA, CITC-DTPA, and cyclohexyl-DTPA.

Claim 47 (Withdrawn): The single molecule reagent according to claim 44, wherein the cyclic amine is selected from the group consisting of NOTA, DOTA, and TETA, and wherein the effector agent comprises a member selected from the group consisting of In, Y, Pb, Bi, Cu, Sm, and Lu radionuclides.

Claim 48 (Withdrawn): The single molecule reagent according to claim 33, wherein the effector agent comprises a member selected from the group consisting of a positron imaging radionuclide, a therapeutic radionuclide, and a gamma imaging radionuclide.

Claim 49 (Withdrawn): The single molecule reagent according to claim 48, wherein the positron imaging radionuclide comprises a member selected from the group consisting of F-18, Br-75, Br-76, and I-124.

Claim 50 (Withdrawn): The single molecule reagent according to claim 48, wherein the therapeutic radionuclide comprises a member selected from the group consisting of Y-90, I-131, In-114m, Re-186, Re-188, Cu-67, Sm-157, Lu-177, Bi-212, Bi-213, At-211, and Ra-223.

Claim 51 (Withdrawn): The single molecule reagent according to claim 48, wherein the gamma imaging radionuclide is selected from the group consisting of Tc-99m, In-111 and I-123.

Claim 52 (Withdrawn): The single molecule reagent according to claim 33, wherein the effector agent comprises a compound which can be converted to a photoactive compound.

Claim 53 (Withdrawn): The single molecule reagent according to claim 52, wherein the compound which can be converted to a photoactive compound comprises a member selected from the group consisting of a chromophore compound and a fluorophore compound.

Claim 54 (Withdrawn): The single molecule reagent according to claim 33, wherein the effector agent is coupled to the tri-functional cross-linking moiety without a second linker.

Claim 55 (Previously Presented): The single molecule reagent according to claim 33, wherein the effector agent is coupled to the trifunctional cross-linking moiety by the second linker which comprises a spacer having a length of 1-25 atoms.

Claim 56 (Previously Presented): The single molecule reagent according to claim 33, wherein the effector agent is coupled to the trifunctional cross-linking moiety by the second linker which comprises a spacer having a length of 6-18 atoms.

Claim 57 (Previously Presented): The single molecule reagent according to claim 33, wherein the second linker aids in water solubility.

Claim 58 (Previously Presented): The single molecule reagent according to claim 57, wherein the second linker comprises a hydrogen-bonding atom.

Claim 59 (Previously Presented): The single molecule reagent according to claim 58 wherein the second linker comprises ethers or thioethers.

Claim 60 (Withdrawn): The single molecule reagent according to claim 57, wherein the second linker comprises an ionizable group.

Claim 61 (Withdrawn): The single molecule reagent according to claim 60, wherein the ionizable group is selected from the group consisting of carboxylates, sulfonates, and ammonium groups.

Claim 62 (Previously Presented): The single molecule reagent according to claim 33, wherein the biomolecule reactive moiety comprises a member selected from the group consisting of an active ester; an N-hydroxy-succinimide ester; a sulfo-N-hydroxysuccinimide ester; a phenolic ester; an aryl imidate; an alkyl imidate; an alkyl isocyanate, an aryl isocyanate or an isothiocyanate which reacts with one or more amino groups on the biomolecule; a maleimide, or an alpha-haloamide which reacts with one or more sulfhydryl groups on the biomolecule; and an arylhydrazine, an alkylhydrazine, an alkyl hydroxylamine or an aryl hydroxylamine which reacts with one or more aldehyde or ketone groups either naturally occurring or synthetically produced on the biomolecule.

Claim 63 (Withdrawn) The single molecule reagent according to claim 33, wherein the biomolecule reactive moiety is coupled to the trifunctional cross-linking moiety without the third linker.

Claim 64 (Previously Presented): The single molecule reagent according to claim 33, wherein the biomolecule reactive moiety is coupled to the trifunctional cross-linking moiety with a third linker comprising a spacer having a length of 1-25 atoms.

Claim 65 (Previously Presented): The single molecule reagent according to claim 33, wherein the biomolecule reactive moiety is coupled to the trifunctional cross-linking moiety with a third linker comprising a spacer having a length of 6-18 atoms.

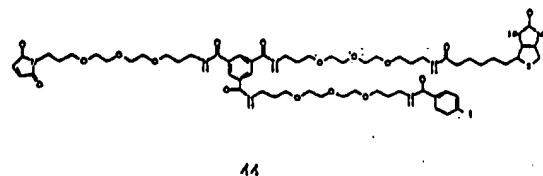
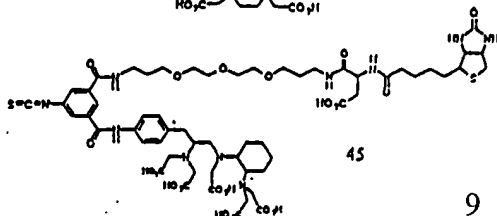
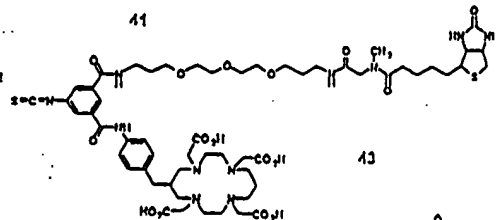
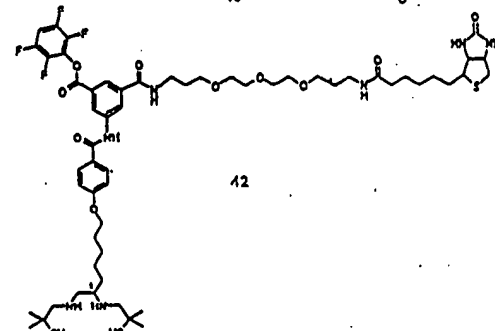
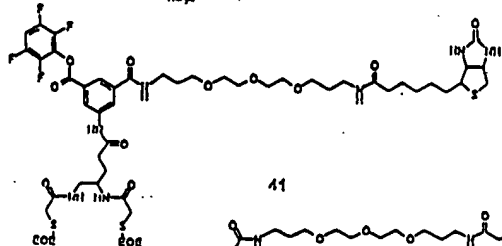
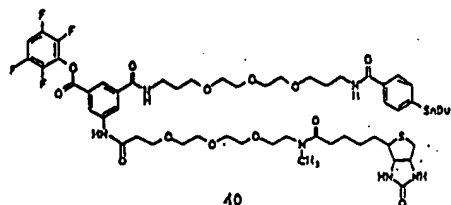
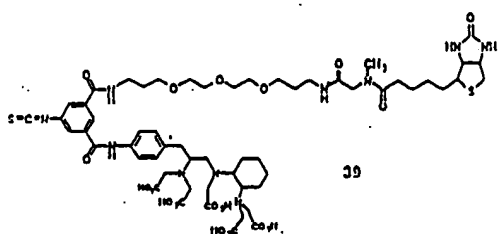
Claim 66 (Previously Presented): The single molecule reagent according to claim 33, wherein the third linker comprises at least one hydrogen-bonding atom.

Claim 67 (Previously Presented): The single molecule reagent according to claim 66, wherein the hydrogen-bonding atom comprises a member selected from the group consisting of ethers and thioethers.

Claim 68 (Withdrawn): The single molecule reagent according to claim 33, wherein the third linker comprises an ionizable group.

Claim 69 (Withdrawn): The single molecule reagent according to claim 68, wherein the ionizable group comprises a member selected from the group consisting of car-boxylates, sulfonates and ammonium groups.

Claim 70 (Currently Amended): The single molecule reagent according to claim 33, wherein the reagent is a member selected from the group consisting of the following compounds:



wherein compounds 41, 42, and 44 are stabilized against enzymatic cleavage by biotinidase.

Claim 71 (Withdrawn): The single molecule reagent according to claim 33, wherein more than one affinity ligand is bound to the trifunctional cross-linking moiety, which is tetrafunctional.

Claim 72 (Withdrawn): The single molecule reagent according to claim 33, wherein more than one effector agent is bound to the trifunctional cross-linking moiety, which is tetrafunctional.

Claim 73 (Previously Presented): A reagent for the diagnosis of a condition or disease in a mammal, the condition or disease selected from the group consisting of cancer, myocardial infarction, deep vein thrombosis, stroke loci, pulmonary embolism and atherosclerosis, the reagent comprising the single molecule reagent according to claim 33.

Claim 74 (Previously Presented): A reagent for the treatment of a condition or disease in a mammal, the condition or disease selected from the group consisting of cancer, myocardial infarction, deep vein thrombosis, stroke loci, pulmonary embolism and atherosclerosis, the reagent comprising the single molecule reagent according to claim 33.

Claim 75 (Withdrawn): A method of detecting an affinity label bound to a biomolecule, comprising:

labeling the biomolecule with biotin or a derivative thereof having a similar

affinity by conjugation of the single molecule reagent according to claim 33, the reagent comprising an affinity ligand having affinity for biotin or a derivative thereof and a detectable effector agent; and

detecting the amount of affinity ligands of the reagent conjugated to the biomolecule.

Claim 76 (Withdrawn): A method for diagnosing a condition or disease in a mammal, comprising:

conjugating a biomolecule to the reagent according to claim 33 to -obtain a conjugated biomolecule;

administering the conjugated biomolecule to the blood circulation of a mammal in need of such diagnosis, such that the conjugated biomolecule is concentrated at a target site at which the reagent is to be detected;

optionally removing from the blood circulation of the mammal any amount of conjugated biomolecule not concentrated at the target site to be detected, by at least one method selected from the group consisting of:

administering to the mammal a protein which specifically binds to the affinity ligand, and

passing the mammalian blood or plasma from the mammal through an affinity column which specifically adsorbs the conjugated biomolecule by specific interaction with the affinity ligand; and

detecting the intended target site specific for the conjugated biomolecule.

Claim 77 (Withdrawn): A method for treating a condition or disease in a mammal, comprising:

conjugating a biomolecule to the reagent according to claim 33 to -obtain a conjugated biomolecule;

administering the conjugated biomolecule to the blood circulation of a mammal in need of such treatment, such that the conjugated biomolecule is concentrated at a site at which the conjugated biomolecule is to exert a therapeutic action; and

optionally removing from the blood circulation of the mammal any amount of such conjugated biomolecule not concentrated at the site at which the reagent is to exert its therapeutic action, by at least one method selected from:

administering to the mammal a protein which specifically binds to the affinity ligand, or

passing the mammalian blood or plasma from the mammal through an affinity column which specifically adsorbs the conjugated biomolecule by specific interaction with the affinity ligand.

Claim 78 (Withdrawn): A method for diagnosing a condition or disease in a mammal, comprising:

conjugating a biomolecule -to the reagent according to claim 33 to -obtain a conjugated biomolecule, wherein the reagent is provided with a radionuclide either before or after conjugation of the biomolecule to the reagent;

administering the conjugated biomolecule to the blood circulation of a mammal in need of such diagnosis, such that the conjugated biomolecule is concentrated at a target site at which the reagent is to be detected;

optionally removing from the blood circulation of the mammal any amount of conjugated biomolecule not concentrated at the target site to be detected, by at least one method selected from the group consisting of:

administering to the mammal a protein which specifically binds to the affinity ligand, and

passing the mammalian blood or plasma from the mammal through an affinity column which specifically adsorbs the conjugated biomolecule by specific interaction with the affinity ligand; and

detecting the intended target site specific for the conjugated biomolecule.

Claim 79 (Withdrawn): A method for treating a condition or disease in a mammal, comprising:

conjugating a biomolecule and a radionuclide -to the reagent according to claim 33 to obtain a conjugated biomolecule, wherein the reagent is provided with a radionuclide either before or after conjugation of the biomolecule to the reagent;

administering the conjugated biomolecule to the blood circulation of a mammal in need of such treatment, such that the conjugated biomolecule is concentrated at a site at which the conjugated biomolecule is to exert a therapeutic action; and

optionally removing from the blood circulation of the mammal any amount of such conjugated biomolecule not concentrated at the site at which the reagent is to exert its therapeutic action, by at least one method selected from:

administering to the mammal a protein which specifically binds to the affinity ligand, or

passing the mammalian blood or plasma from the mammal through an affinity column which specifically adsorbs the conjugated biomolecule by specific interaction with the affinity ligand.

Claim 80 (Withdrawn): A method of detecting an affinity label bound to a biomolecule, comprising:

labeling a biomolecule with an affinity label by conjugation of the reagent according to claim 33 to obtain an affinity-labeled biomolecule; and
determining an activity and thereby an amount of the effector agent of the reagent, wherein an amount and activity of the effector agent is proportional to the number of affinity ligands on the biomolecule.

Claim 81 (Withdrawn): A kit for diagnosing a condition or disease in a vertebrate host, comprising:

- a diagnostic biomolecule;
- the single molecule reagent according to claim 33;
- an optional plasma separation device for separation of plasma from blood;
- optional means for extra-corporeal circulation of whole blood or plasma from the vertebrate host; and
- an optional extracorporeal adsorption device comprising immobilized receptors specific toward the affinity ligand of the single molecule reagent.

Claim 82 (Withdrawn): A kit for treating a condition or disease in a vertebrate host, comprising:

- a therapeutic biomolecule;
- the single molecule reagent according to claim 33;
- an optional plasma separation device for separation of plasma from blood;
- optional means for extra-corporeal circulation of whole blood or plasma from the vertebrate host; and
- an optional extracorporeal adsorption device comprising immobilized receptors specific towards the affinity ligand of the single molecule reagent.

Claim 83 (Withdrawn): The kit according to claim 81, wherein the effect-or agent of the single molecule reagent is selected from the group consisting of radionuclide binding/bonding moieties with or without the radionuclide.

Claim 84 (Withdrawn): The kit according to claim 82, wherein the effect or agent of the single molecule reagent is selected from the group consisting of synthetic or naturally occurring toxins, enzymes capable of converting a prodrug to an active drug, immunosuppressive agents, immunostimulating agents, and radionuclide binding/bonding moieties with or without the radionuclide.

Claim 85 (Withdrawn): The kit according to claim 81, wherein the affinity ligand comprises biotin, or a biotin derivative having essentially the same binding function to avidin or streptavidin as biotin, and wherein the immobilized receptor comprises a member selected from the group consisting of avidin; a derivative, mutant or fragment of avidin having essentially the same binding function to the affinity ligand as avidin; streptavidin; and a derivative, mutant or fragment of streptavidin having essentially the same binding function to the affinity ligand as streptavidin.

Claim 86 (Withdrawn): The kit according to claim 82, wherein the affinity ligand comprises biotin, or a biotin derivative having essentially the same binding function to avidin or streptavidin as biotin, and wherein the immobilized receptor comprises a member selected from the group consisting of avidin; a derivative, mutant or fragment of avidin having essentially the same binding function to the affinity ligand as avidin; streptavidin; and a derivative, mutant or fragment of streptavidin having essentially the same binding function to the affinity ligand as streptavidin.

Claim 87 (Withdrawn): A method of using the kit according to claim 81, comprising:

conjugating the single molecule reagent with the diagnostic biomolecule to obtain a conjugated biomolecule;

adding the conjugated biomolecule to the blood circulation of the vertebrate host, such that the conjugated biomolecule is concentrated at a target site to be detected;

optionally extra-corporeally circulating the whole blood or plasma from the vertebrate host by means of the means for extra-corporeal circulation;

optionally separating the plasma from the blood by means of the plasma separation device; and

optionally adsorbing any conjugated biomolecule in the blood or plasma by means of the optional extracorporeal adsorption device.

Claim 88 (Previously Presented): A diagnostic or therapeutic conjugate which can be extracorporeally eliminated from the blood of a mammal to which it is administered to minimize undesired toxic side-effects, the conjugate comprising:

a biomolecule, and

the single molecule reagent according to claim 33, having at least one affinity ligand and at least one effector agent bound to the reagent.

Claim 89 (Previously Presented): A method of making a diagnostic or therapeutic conjugate which can be extracorporeally eliminated from the blood of a mammal to which it is administered to minimize undesired toxic side-effects, the method comprising reacting:

a biomolecule, and

the single molecule reagent according to claim 33, having at least one affinity ligand bound and at least one effector agent bound to the reagent.

Claim 90 (Withdrawn): The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises aminoisophthalic acid;
the affinity ligand comprises biotin;
the second linker comprises an aminobenzyl group; and
the biomolecule reactive moiety comprises an isothiocyanate.

Claim 91 (Withdrawn): The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises aminoisophthalic acid;
the affinity ligand comprises biotin;
the second linker comprises a trioxadiazine; and
the biomolecule reactive moiety comprises a tetrafluorophenyl ester.

Claim 92 (Withdrawn): The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises aminoisophthalic acid;
the affinity ligand comprises homobiotin;
the first linker comprises a trioxadiazine;
the second linker comprises a propionate moiety; and
the biomolecule reactive moiety comprises a tetrafluorophenyl ester.

Claim 93 (Withdrawn): The single molecule reagent according to claim 33, wherein:

the trifunctional cross-linking moiety comprises aminoisophthalic acid;
the affinity ligand comprises homobiotin;
the first linker comprises a trioxadiazine;
the second linker comprises a pentyloxybenzoate group; and
the biomolecule reactive moiety comprises a tetrafluorophenyl ester.

Claim 94 (Withdrawn): The single molecule reagent according to claim 33, wherein:

- the trifunctional cross-linking moiety comprises aminoisophthalic acid;
- the affinity ligand comprises biotin;
- the first linker comprises a biotinidase-stabilizing linker;
- the second linker comprises an aminobenzyl group; and
- the biomolecule reactive moiety comprises an isothiocyanate.

Claim 95 (Withdrawn): The single molecule reagent according to claim 33, wherein:

- the trifunctional cross-linking moiety comprises tricarboxybenzene;
- the affinity ligand comprises biotin;
- the first linker comprises a biotinidase-stabilizing linker;
- the second linker comprises a trioxadiazine moiety;
- the third linker comprises a trioxadiazine moiety; and
- the biomolecule reactive moiety comprises a maleimide group.

Claim 96 (Withdrawn): The single molecule reagent according to claim 33, wherein:

- the trifunctional cross-linking moiety comprises aminoisophthalic acid;
- the affinity ligand comprises biotin;
- the first linker comprises a biotinidase-stabilizing linker;
- the second linker comprises an aminobenzyl group; and
- the biomolecule reactive moiety comprises an isothiocyanate.

Claim 97 (Withdrawn): A method for the detection of affinity ligand bound to a biomolecule, comprising

labeling the biomolecule with biotin or a derivative thereof having a similar affinity, by conjugation of the single molecule reagent according to claim 33, the reagent comprising an affinity ligand having affinity for biotin or a derivative thereof; and
detecting an amount of affinity ligand of the reagent conjugated to the biomolecule.

Claim 98 (New): The single molecule reagent according to claim 33, wherein the N-methyl group is introduced through the introduction of an N-methyl glycyl residue in linker 1.